



UNIVERSITI PUTRA MALAYSIA

**MICROEMULSION WITH ESTER IN MIXED SURFACTANTS AND
ITS ASSOCIATION WITH BETULINIC ACID**

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**MICROEMULSION WITH ESTER IN MIXED SURFACTANTS AND
ITS ASSOCIATION WITH BETULINIC ACID**

By

WAN RUSMAWATI BT WAN MAHAMOD

**Thesis Submitted in Fulfilment of the Requirements for
the Degree of Master of Science in the Faculty of
Science and Environmental Studies
Universiti Putra Malaysia**

February 2000



CHAPTER 1

INTRODUCTION

A surface active agent, or 'surfactant', is a chemical compound with a polar or ionic head and a hydrocarbon tail (Figure 1) (Ottewil, 1983; Benson, 1983; Lucassen, 1981). The hydrocarbon tail is hydrophobic and can be either linear or branched. The polar, or ionic, head is hydrophilic, interacting strongly with the water molecules with hydrogen bonding and dispersing them via dipole-dipole or ion-dipole interaction. A structure having both a hydrophobic and hydrophilic moieties is said to be 'amphiphatic'.

Surfactants are classified by their hydrophilic heads into four types - ionic (either cationic or anionic) and nonionic (either zwitterionic or amphoteric) (Figure 2).

The balance between the hydrophobic and hydrophilic portions of a surfactant confers it its unique properties in use (Karsa, 1987; Myers, 1990). The applications of surfactants are many, in both everyday household products and personnel care, and in the industrial production and processing of materials (Figure 3).

*Dedicated to
In memory of my father*

*"Thank you for your everlasting love and support.
Although you are not able to share my success
I know that you will be very happy for me."*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirements for the degree of Master of Science.

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February 2000

Chairman : Assoc. Prof. Dr. Faujan Bin Haji Ahmad

Faculty : Science and Environmental Studies

The phase behaviour of Tween 80/Brij 30/H₂O and Tween 80/Brij 30/glycerol was constructed to determine the isotropic region produced by the mixed surfactants (MS) – Tween 80:Brij 30 - in aqueous and nonaqueous systems. The weight ratios of 90:10, 80:20 and 60:40 Tween 80:Brij 30 were selected for further construction of MS systems of MA or EB/Tween 80:Brij 30/water and MA or EB/Tween 80:Brij 30/glycerol. MS in aqueous systems produced a larger isotropic region than the single phase MA or EB/Tween 80/water and MA or EB/Brij 30/water systems. The system of MA/Tween 80:Brij 30 (90:10, 80:20 and 60:40)/water was then studied to determine the surfactants' aggregation and their association with BA.

The conductivity was measured to assess the charge carrier and the movement of ions in the system. The structure of the isotropic region was also determined. A reverse micelle (w/o micromulsion), bicontinuous structure

and micelle (o/w microemulsion) formed in the range of 20 – 40wt%, 40 – 60wt% and >60wt% water, respectively. However, the MA/Tween 80:Brij 30 (80:20)/water system did not form a micelle. The results were confirmed by light scattering measurement in which the particle size in each region was determined.

BA was more soluble in the system with higher Tween 80. The solubility of BA increased with the MA content and decreased with the water content.

In the antimicrobial study, 0.01 wt% BA in the microemulsion MA/Tween 80:Brij 30 (80:20)/water was more active against *Candida lipolytica* ATCC 2075 than the pure microemulsion itself. A higher mixed surfactant content (20:80 weight ratio of MA and MS) gave better activity than a lower one (50:50 weight ratio). BA in the reverse micelle region gave the highest activity compared to in the monomer and bicontinuous regions.

The cytotoxicity study produced unexpected results. The microemulsion systems were very active and killed all the cells (CEM-SS) leaving no control data with which to assess the cytotoxicity.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk syarat ijazah Master Sains

**MIKROEMULSI DENGAN ESTER DI DALAM CAMPURAN
SURFAKTAN DAN GABUNGANNYA DENGAN ASID BETULINIK**

Oleh

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Gambarajah fasa Tween 80/Brij 30/air dan Tween 80/Brij 30/gliserol dibina bagi menentukan kawasan isotropik yang terhasil dari campuran Tween 80:Brij 30 dalam akuas dan nonakuas. Nisbah berat antara Tween 80 dan Brij 30 pada 90:10, 80:20 and 60:40 dipilih bagi pembinaan gambarajah fasa sistem MA atau EB/Tween 80:Brij 30/air and MA atau EB/Tween 80:Brij 30/gliserol. Campuran surfaktan dalam system akuas menghasilkan kawasan isotropik yang lebih luas berbanding dengan gambarajah fasa tunggal, MA atau EB/Tween 80/air dan MA atau EB/Brij 30/air. Sistem MA/Tween 80:Brij 30 (90:10, 80:20 and 60:40)/air kemudiannya digunakan dalam kajian lanjutan agregasi surfaktan yang terhasil serta gabungannya dengan BA.

Konduktiviti diukur bagi menentukan pembawaan cas dan pergerakan ion di dalam sistem. Struktur bagi kawasan isotropik juga telah ditentukan. Misel songsang, misel campuran dan misel normal masing-masing wujud

dalam julat 20 – 40%, 40 – 60% dan >60% berat air. Walau bagaimanapun, sistem MA/Tween 80 :Brij 30 (80:20)/air tiada pembentukan misel. Keputusan diperkukuhkan lagi melalui pengukuran sebaran cahaya di mana saiz partikel di dalam setiap kawasan ditentukan.

BA lebih larut di dalam sistem yang mengandungi peratusan Tween 80 yang tinggi. Kelarutan BA meningkat dengan peningkatan komposisi MA dan menurun dengan peningkatan komposisi air.

Di dalam kajian antimikrob, 0.01% berat BA di dalam sistem mikroemulsi MA/Tween 80:Brij 30 (80:20)/air paling aktif terhadap *candida lipolytica* ATCC 2075 berbanding dengan sistem mikroemulsi tersebut. Sistem yang mengandungi campuran surfaktan yang tinggi (nisbah berat MA dan campuran surfaktan pada 20:80) adalah lebih aktif berbanding dengan sistem yang rendah komposisi campuran surfaktan (nisbah berat MA dan campuran surfaktan pada 50:50). BA di dalam misel songsang memberikan aktiviti yang paling baik berbanding dengan kawasan monomer dan misel campuran.

Kajian sitotoksik menghasilkan keputusan diluar jangkaan. Sistem mikroemulsi sangat aktif dan telah membunuh semua sel CEM-SS tanpa meninggalkan kontrol bagi penilaian kesan sitotoksik.

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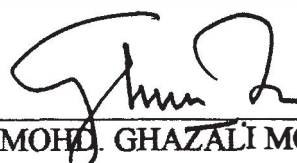
I certify that an Examination Committee met on 26th February 2000 for the final examination of Wan Rusmawati Bt Wan Mahamod on her Master of Science thesis, "Microemulsion with Ester in Mix Surfactants and its Association with Betulinic Acid", in accordance with the Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or at any other institution.



(Wan Rusmawati Bt. Wan Mahamod)

Date: 22/5/2000

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LIST OF ABBREVIATIONS

BA	Betulinic acid
MA	Methyl acetate
H ₂ O	Deionised water
MS	Mixed surfactants
DMSO	Dimethyl sulphoxide
ATCC	American Type Culture Collection
NRRL	Northern Regional Research
EB	Ethyl benzoate
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
NA	Nutrient Agar
NB	Nutrient Broth
MTT	Micro-culture Tetrazolium Salt
W/O	water-in-oil
O/W	oil-in-water
OD	Optical Density
CEM-SS	T-Lymphoblastic Leukemia cell
IC ₅₀	Inhibitory Concentration at 50% cells reduction
L	Lamellar liquid crystal
D	Cubic liquid crystal
E	Hexagonal liquid crystal
L ₁	Normal micelle (o/w microemulsion)

L_2	Reverse micelle (w/o microemulsion)
HLB	Hydrophilic Lipophilic Balance
T	Two-phase region

CHAPTER 1

INTRODUCTION

A surface active agent, or 'surfactant', is a chemical compound with a polar or ionic head and a hydrocarbon tail (Figure 1) (Ottewil, 1983; Benson, 1983; Lucassen, 1981). The hydrocarbon tail is hydrophobic and can be either linear or branched. The polar, or ionic, head is hydrophilic, interacting strongly with the water molecules with hydrogen bonding and dispersing them via dipole-dipole or ion-dipole interaction. A structure having both a hydrophobic and hydrophilic moieties is said to be 'amphiphatic'.

Surfactants are classified by their hydrophilic heads into four types - ionic (either cationic or anionic) and nonionic (either zwitterionic or amphoteric) (Figure 2).

The balance between the hydrophobic and hydrophilic portions of a surfactant confers it its unique properties in use (Karsa, 1987; Myers, 1990). The applications of surfactants are many, in both everyday household products and personnel care, and in the industrial production and processing of materials (Figure 3).

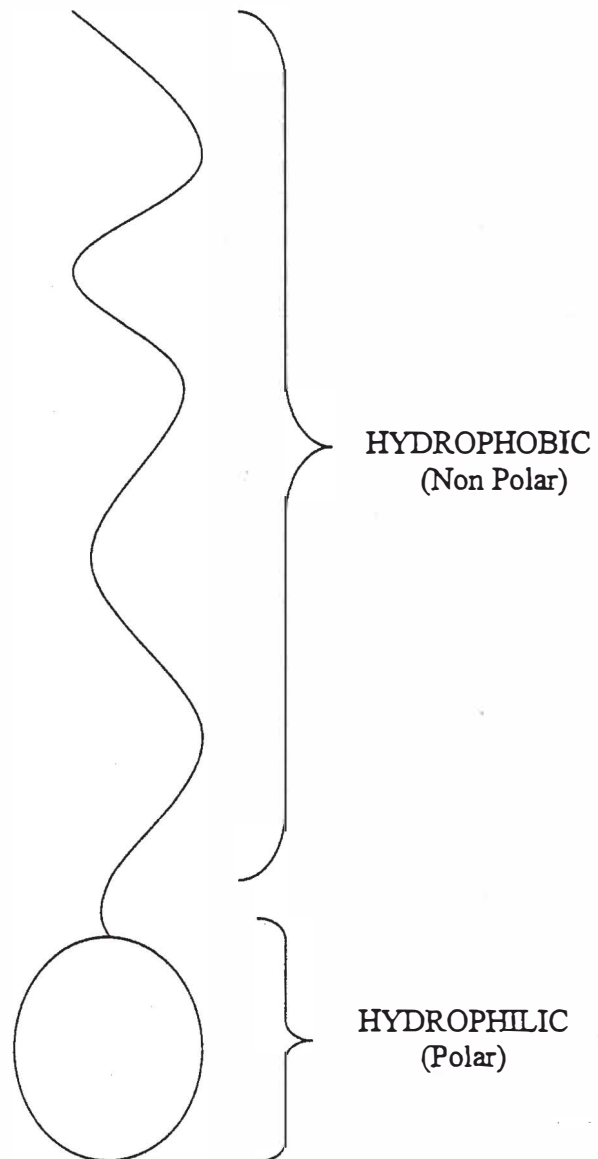


Figure 1: Structure of a surfactant